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Wine and health: evidences and mechanisms

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Abstract

Research on wine and health during the last decade has been very productive. Epidemiologically, it is generally accepted today that non-drinkers, as well as excessive drinkers ("J curve") have higher incidence of cardiovascular disease, as well as other chronic diseases, and live longer. In many studies wine appears as the most effective alcoholic beverage, but also some studies support the role of alcohol and either, do not reveal a difference with wine, or the role of wine appears confounded with the better lifestyle of wine drinkers.

Scientifically, after the epidemiologists recognize the effect of wine, the challenge is to find the biological basis of the phenomena. The research has focused on alcohol and on phenolics as wine components, and on several biological targets, recognized as cardiovascular risk factors, that would be modified in the consumers.

The proposed mechanisms mediating the effect of ethanol are: increased HDL levels, decreased fibrinogen, increases in plasminogen and t-PA, eNOS stimulation, and a direct cardioprotective effect on the heart.

The phenolics present in wine belong to a large class of compounds and their active metabolites, all of them endowed with several biological activities which could be involved in the protective effects of wine. Interestingly, some effects attributed exclusively to ethanol like HDL elevation, decreased thrombus formation and alcohol mediated preconditioning, are all closely related to free radical metabolism and oxidative stress. The same is true for endothelial function.

Decreased cardiovascular disease and longevity are epidemiological parameters associated to wine consumption. Recent evidence suggest that longevity, in addition to decreased CV disease, could also be the direct consequence of phenolics activating histone deacetylation, a gene expression regulatory mechanism proposed to explain the longevity associated to caloric restriction.

Epidemiological observations give us valuable insights in the role played by the environment on human health. The observations lead to hypotheses which necessarily have to be confronted in the laboratory. This sequence is apparent in the development of our present ideas related to wine and health. The epidemiological work is not finished and much remains to be evaluated experimentally.

Epidemiological evidences

Epidemiological studies show that moderate alcohol consumption, particularly wine, is associated to decreased risk in all-causes mortality, especially ischemic heart disease death as well as other chronic diseases, and a longer life [1, 2]. The shape of the correlation graphic is generally described as a J-shaped curve in which moderate drinkers (two drinks per day) of any kind of alcohol have a decreased risk of coronary disease relative to non-drinkers, whereas heavy drinkers have an increased risk of infarction and stroke.

Moderate drinkers, male or female, show a 30 to 40% decrease in risk of coronary heart disease, and 10 to 20% decrease in mortality from all causes. On cardiovascular disease a reduction which might reach 60% in morbidity and mortality has been reported [3-8].

The studies and discussions held on the so called "French Paradox" give to wine a special position. It is a characteristic component of the Mediterranean diet and might account for the lower incidence of coronary heart disease among Mediterranean populations [9-10]. Renaud and Ruf show that the correlation among coronary deaths and various foods, in 21 countries, is stronger for wine (-0.87, $p<0.001$) than for other components like vegetables or vegetable fats; at the same time they found a positive correlation with milk-fat products (0.66, $p<0.001$). Thus, wine would exert a more marked effect than that of fruits and vegetables, also rich in antioxidants [10]. The same general conclusions were reached for alcohol, most particularly wine, in a study which involved countries with similar economical development [11].

Gronbaek and collaborators in the Copenhagen City Heart Study on 6051 men and 7234 women, 30 to 79 years old, found that wine, but not beer or spirits, was associated to a

decrease in cardiovascular, cerebrovascular, and all causes mortality [12]. In this study, 3 to 5 drinks per day led to reduction in cardiovascular mortality by 47% in the wine drinkers, and also mortality by other causes was 50% lower. This work is in contrast to others in which not only wine, but also other alcoholic beverages are considered to be responsible for the beneficial effects [13]. A more recent work by Renaud and collaborators on 34,014 middle-aged men from eastern France, 77% of them wine drinkers, led to the conclusion that moderate consumers (2 to 5 glasses per day) have a 24-31% reduction in all-cause mortality, attributed to cardiovascular disease and cancer [6, 14]. The results also showed that the protective effect of a moderate intake of wine on all-cause mortality is observed at all levels of blood pressure and serum cholesterol.

The specificity of wine for lowering the risk of all-cause mortality and cancer has been confirmed by Gronbaek and collaborators [15]. It was only in wine drinkers that the all-cause mortality was lowered by more than 20% for an intake of up to 3 drinks per day. They also found that it was only wine but not spirits or beer that reduced cancer mortality by up to 20% (3 glasses of wine/day). As for cancer, the subsequent question was to evaluate on what types of cancer wine may have protective effects. Gronbaek and collaborators have already shown that wine drinking does not increase the risk of upper digestive tract cancer as compared to beer or spirits [16].

Moderate wine or alcohol consumption is also beneficial for conditions associated to aging. A better cognitive performance and lower risk of dementia have been described [17-18].

However, some studies showed a difference according to the type of alcohol. In the Canadian Study of Health and Aging, the follow up of 4088 persons for 5 years showed a 31% reduction of the risk of developing dementia in alcohol drinkers compared to non drinkers [19]. In addition, the risk was lower in wine drinkers (odds ratio (OD) 0.49; 95% confidence interval (CI) 0.28-0.88) than in beer or spirit drinkers (OR 0.84; 95% CI 0.51-1.41 and OR 0.78; 95% CI 0.52-1.19), respectively.

In another cohort (the Copenhagen City Heart Study), the alcohol consumption recorded in 1976 in a cohort initially designed to study cardiovascular diseases has been linked to the risk of getting dementia in the period 1990-94 [20]. Compared to non drinkers, the risk of developing dementia was significantly lower among occasional wine drinkers (OR 0.43; 95% CI 0.23-0.82), in weekly drinkers (OR 0.33; 95% CI 0.13-0.86), but non significant in daily drinkers (OR 0.57; 95% CI 0.15-2.11). However, an increased risk was observed for beer (OR 2.28; 95% CI 1.13-4.60 in occasional drinkers, OR 2.15; 95% CI 0.98-4.78 in weekly drinkers and OR 1.73; 95% CI 0.75-3.99 in daily drinkers) or for spirits (OR 0.81; 95% CI 0.42-1.57 in occasional drinkers, OR 1.65; 95% CI 0.74-3.69 in weekly drinkers and OR 1.12; 95% CI 0.43-2.92 in daily drinkers).

In Bordeaux (France), a population-based prospective study found that subjects drinking 3 to 4 standard glasses of wine per day (> 250 and up to 500 ml), categorized as moderate drinkers, the crude OR was 0.18 for the incidence of dementia ($p < 0.01$) and 0.25 for Alzheimer's disease (AD)($p < 0.03$), as compared to the non-drinkers. After adjusting for age, sex, education, occupation, baseline cognitive performances and other possible confounders, the ORs were respectively 0.19 ($p < 0.01$) and 0.28 ($p < 0.05$). In the 922 mild drinkers (< 1 to 2 glasses per day) there was a negative association only with AD, after adjustment (OR 0.55; $p < 0.05$). The inverse relationship between moderate wine drinking and incident dementia was explained neither by known predictors of dementia nor by medical, psychological or socio-familial factors [21-22].

A recent cohort of elderly persons from New York City was studied, 980 community-dwelling individuals aged 65 and older without dementia at baseline and with data on alcohol intake recruited between 1991 and 1996 and followed annually. After 4 years of follow-up, 260 individuals developed dementia (199 AD, 61 dementia associated with stroke). After adjusting for age, sex, apolipoprotein E (ApoE)-epsilon 4 status, education, and other alcoholic beverages, only intake of up to three daily servings of wine was associated with a lower risk of AD (OD 0.55; 95% CI 0.34-0.89). Intake of liquor, beer, and total alcohol was not associated with a lower risk of AD. Stratified analyses by the ApoE-

epsilon 4 allele revealed that the association between wine consumption and lower risk of AD was confined to individuals without the APOE-epsilon 4 allele [23].

Diabetes (NIDDM) is another condition that correlates with aging and in which oxidative stress plays a pathogenic role. Moderate consumers have a decreased risk of diabetes [24]. Macular degeneration is also an age-related disorder in which moderate wine consumption correlates with decreased risk [25].

Moderate wine drinkers appear to be at lower risk of becoming heavy and excessive drinkers as well as developing alcoholic cirrhosis [26-27]. This study suggests that a person who prefers beer is more likely to become a heavy or an excessive drinker than a person who prefers wine. Among men, moderate drinkers who included wine in their weekly alcohol intake had significantly lower risks of becoming heavy or excessive drinkers as compared to those who did not drink any wine. Women who included beer in their alcohol intake showed increased risk of heavy and excessive drinking compared to non-beer drinking women. Further they found that the risk of developing cirrhosis in wine drinkers (more than 30% wine in their total alcohol intake) was less than 50% of the risk in non-wine drinkers for any given level of total alcohol intake, while beer and spirits drinking did not modify the relation between total alcohol intake and risk of developing cirrhosis.

Other studies have reported relations between beer drinking and high-risk behaviors such as frequent heavy drinking and other alcohol-related problems, while wine drinking seems to be considered 'the beverage of moderation' [28-30].

There is a large variety of other positive phenomena associated to moderate wine consumption. Wine has been described as a healthy beverage for centuries, a property mainly associated to its capacity of killing or stopping the growth of microorganisms. This has been observed for many bacteria and there is an interesting report on the capacity of wine to control oyster-born hepatitis A [31]. A particularly intriguing property of wine is that in contrast to beer and hard liquor, it does not favor an increase in the waist-to-hip ratio, a parameter strongly associated to cardiovascular risk [32]. The risk of kidney stones

also decreases in moderate wine consumers, in studies that simultaneously show that fruit juices, particularly grape-fruit juice, increase the risk [33].

Deleterious effects of alcohol consumption

When dealing with the negative effects of alcoholic beverage consumption it is necessary to establish very clearly the levels of consumption that correspond to a statement such as "alcohol consumption leads to cardiovascular morbidity, to cirrhosis, to birth defects, to cancer, to hypertension, and to migraine". Indeed the very concept of moderate consumption stems, among others, from studies that identify the consumption levels associated to minimal risk. There have been discussions on the meaning of the increased cardiovascular risk of non-drinkers, yet the meaning of the increased risk for heavy drinkers is straight forward, but often forgotten by those who stress cirrhosis as the consequence of excessive drinking.

A positive correlation has been observed for hemorrhagic stroke and drinking, however this effect has to be confronted with the higher frequency of obstructive events that do benefit from moderate consumption [1].

There are a number of cancers that are referred to as alcohol-related; cancers of the mouth, pharynx, larynx, esophagus, and sometimes stomach and liver. They are associated with very heavy drinking, with alcohol abuse; generally, they correlate with heavy smoking and heavy drinking, and do not show increased frequency in light-to-moderate drinkers. Gronbaek and collaborators in a population cohort study observed the association between alcohol intake and cancer of the upper digestive tract [16]. Their conclusion was that a moderate intake of wine probably does not increase the risk, whereas moderate intake of beer or spirits increases the risk considerably. In an editorial commentary, the possible role of nitrosamines is emphasized. For lung cancer, in three prospective Danish studies, it was found that a high consumption of beer and spirits is associated to an increased risk of lung cancer, whereas wine intake apparently protects [34]. For mammary cancer no clear demonstration of alcohol beverages as risk factor was found [35]. Yet, a significant correlation for women drinking 3 or more glasses per day has been described [4] and in

Mediterranean populations, with a much lower breast cancer incidence than in USA, have been found a doubling in the frequency for women drinking two to three glasses per day [36]. The conclusion is that with the possible exception of breast cancer, moderate drinkers are not at increased risk of any type of cancer [37].

Wine should be considered as a healthy component of the diet in many cultures. On the whole, with the evidence available, moderate wine consumption should be considered as safe, with the sole exception of activities requiring a maximum degree of alertness. It requires further research the hypothesis that genetically predisposed people might become addict to alcohol by moderate consumption [38].

Epidemiological conclusions

Today the main concern for most epidemiologists is not the benefits, which are not contested, but the potential negative consequences associated to moderate drinking.

Additionally, some epidemiologists reject a difference among wine and other alcoholic beverages. The differences are attributed to lifestyle differences among those who prefer one type of alcoholic beverage over another thus making it exceedingly difficult to determine whether the differences in apparent health effects are actually related to the beverage itself. Drinkers of any type of wine have a lower mortality risk than do beer or liquor drinkers, yet it remains unclear whether this reduced risk is due to nonalcoholic wine ingredients, drinking patterns or associated traits [39].

Mechanisms

Biology of wine constituents: effects of alcohol and polyphenols

After the epidemiologists recognize the potential role of wine, the challenge is to find the biological basis of the phenomena. So far the research has focused on alcohol and on phenolics as wine components, and on several biological targets, recognized as cardiovascular risk factors, that would be modified in the consumers.

Alcohol

Alcohol intake modifies plasma lipids and haemostatics factors. The health related biological effects observed in response to ethanol consumption are: increased high density lipoprotein (HDL) cholesterol levels, decreased fibrinogen, increased plasminogen and tissue type plasminogen activator (t-PA), endothelial nitric oxide synthase (eNOS) stimulation, and a direct cardioprotective effect on the heart.

Lipid factors

A high concentration of serum cholesterol is a major risk factor for coronary heart disease (CHD). This risk is mediated through the major cholesterol-carrying lipoprotein of serum, low-density lipoprotein (LDL). Additionally, decreased HDL cholesterol levels constitute a central risk factor for CHD [40].

At the present there is consensus on the positive association between alcohol intake and plasma HDL cholesterol level. From some studies it is estimated that half of the beneficial effect of moderate alcohol intake is due to increased HDL cholesterol concentration. Rimm and collaborators in a meta-analysis reported that an experimental dose of 30 g of ethanol a day increased LDL cholesterol by 3.99 mg/dl (95% CI 3.25-4.73) [41]. There are also evidences that alcohol dehydrogenase genotype and alcohol metabolic rate does not modify the effects of alcohol on plasma HDL concentration [42].

One of the main antiatherogenic functions of HDL is reverse cholesterol transport. HDL removes unesterified, or “free” cholesterol from peripheral tissues, after which much of the cholesterol is esterified by the plasma enzyme lecithin:cholesterol acyltransferase. Subsequently, HDL cholesterol is efficiently delivered directly to the liver and steroidogenic tissues via a selective uptake pathway [43]. Moderate alcohol intake increases serum HDL cholesterol level and stimulates cellular cholesterol efflux [44]. Exercise also increases serum HDL cholesterol level as improves reverse cholesterol transport [45].

Human serum paraoxonase, an esterase, is associated with HDL and has been shown to reduce the susceptibility of LDL to lipid peroxidation [46]. As the oxidative modification of LDL plays a central role in the initiation and acceleration of atherosclerosis, increased

serum levels of paraoxonase in HDL diminish the atherogenic effect of LDL [47]. There are evidences that light drinking upregulates, whereas heavy drinking downregulates paraoxonase activity and its expression, irrespective of its genetic polymorphism in rats and humans [48].

The HDL receptor SR-BI (scavenger receptor class B type I) mediates the selective uptake of plasma HDL cholesterol by the liver and steroidogenic tissues. As a consequence, SR-BI can influence plasma HDL cholesterol levels, HDL structure, biliary cholesterol concentrations, and the uptake, storage, and utilization of cholesterol by steroid hormone-producing cells [49]. This receptor also facilitates efficient transfer of vitamin E (alpha-tocopherol) from HDL to cultured cells. In SR-BI-deficient mutant mice, relative to wild-type control animals, there was a significant increase in plasma alpha-tocopherol levels. This increase in plasma alpha-tocopherol was accompanied by a significant decrease (65-80%) in the alpha-tocopherol concentrations in bile and several tissues including ovary, testis, lung and brain but not in the liver, spleen, kidney or white fat. These data show that SR-BI plays an important role in transferring alpha-tocopherol from plasma lipoproteins to specific tissues. Defective tissue uptake of lipoprotein alpha-tocopherol in SR-BI-deficient mice may contribute to the reproductive and cardiovascular pathologies exhibited by these animals [50]. Oxidative stress regulates the expression of SR-BI receptor, oxidized LDL (oxLDL) decreased SR-BI expression in a dose- and time-dependent manner and the ability of oxLDL to decrease SR-BI expression was dependent on the degree of LDL oxidation. OxLDL decreased both [(14)C]cholesteryl oleate/HDL uptake and efflux of [(14)C]cholesterol to HDL in a time-dependent manner [51].

Sex is another factor associated with decreased risk of developing cardiovascular disease. Decreased risk is found in premenopausal women, together with elevated HDL levels. HDL and estrogen stimulate eNOS and the production of nitric oxide (NO) which has numerous protective effects in the vascular system, including vasodilation, reduced leukocyte adhesion, and anti-inflammatory effects [52]. HDL isolated from premenopausal women, or postmenopausal women receiving estradiol replacement therapy, stimulated eNOS; whereas HDL isolated from postmenopausal women or men had minimal activity. HDL-

associated estradiol is capable of stimulating eNOS in an SR-BI-dependent manner [53] leading to a new paradigm that involves eNOS, for the explanation of the cardiovascular effects of HDL and estrogens.

Haemostasis factors

The effects of alcohol on the coagulation and thrombolytic processes are not sufficiently studied. In general, haemostatic factors in moderate alcohol drinkers show a more thrombolytic profile. Consistent evidence has been provided linking moderate alcohol intake with lower fibrinogen levels. Rimm and collaborators, in the meta-analysis reported that 30g of alcohol a day was associated with a 7.5 mg/dl decrease in fibrinogen concentration [41].

Numerous studies have investigated the effect of alcohol on platelet aggregation. All tend to demonstrate that alcohol added *in vitro* leads to a significant decrease of platelet aggregation induced by thrombin, collagen, epinephrine and ADP [54]. Some human studies have shown that physiological concentrations of ethanol inhibit platelet aggregation in humans as well as in animals in response to several agonists, like collagen, thrombin, ADP and platelet activating factor, in others this inhibition was not found [55-57]. Therefore aggregation studies are not consistent, a finding attributed to the difference on assay methods used *in vitro* or *ex vivo* to measure platelet aggregation [41].

A rebound phenomenon of hyperaggregability is observed after acute alcohol consumption but not after wine consumption. The apparent protection afforded by wine has been replicated in animals with grape phenolics added to alcohol. This rebound phenomenon could explain the ischemic strokes or sudden deaths known to occur after episodes of drunkenness [58].

Fibrinolysis is increased in alcohol drinkers. Rimm and collaborators reported that 30g of alcohol a day was associated with a 1.25 ng/dl increase in t-PA antigen concentration, and a 1.47% increase in plasminogen concentration [41].

Preincubation of human monocytes with low alcohol followed by incubation in the absence of alcohol resulted in an increase in t-PA and urokinase type plasminogen activator (u-PA) expression [59]. And in mice, ethanol induces a significative increase in clot lysis, increases expression for t-PA and u-PA, and decreases expression for PAI-1(plasminogen activator inhibitor) [60].

We carried out an intervention study in humans to evaluate the effect of a Mediterranean diet (MD), an Occidental diet (OD) and their supplementation with red wine, on biochemical, physiological and clinical parameters related to atherosclerosis and other chronic diseases. For 3 months, two groups of 21 male volunteers each received either a MD or an OD; during the second month, red wine was added isocalorically, 240 ml/day. At days 0, 30, 60 and 90, clinical, physiological and biochemical evaluations were made. We founded that MD, compared with OD, was associated with an improvement in haemostatic cardiovascular risk factors: lower plasma fibrinogen and factors VIIc and VIIIc, higher levels of protein S and longer bleeding time. Red wine supplementation of both diets resulted in further decrease in plasma fibrinogen and factor VIIc, and in increases in PAI-1 and t-PA antigen. Red wine was also associated with an increase in antithrombin III (ATIII) but only in individuals on MD. Overall, these findings provide evidence that both MD and red wine have independent but complementary benefits with regard to cardiovascular risk. They decrease thrombosis [57, 61].

The antithrombotic effect of red wine is attributed in part to alcohol, but mainly to wine phenols [62]. Alcohol and polyphenols are both involved, as discussed below.

Other alcohol effects

In rats, moderate alcohol consumption induced significant oxidative stress in the heart which was then reflected into induction of the expression of several cardioprotective oxidative stress-inducible proteins including heat shock protein (HSP) 70. So alcohol by itself imparts cardioprotection by adapting the hearts to oxidative stress, with a reduction of myocardial ischemic reperfusion injury, myocardial infarct size and cardiomyocyte apoptosis [63-64].

Polyphenols

Polyphenol bioavailability

The total amount of phenols found in a glass of red wine is on the order of 200 mg versus about 40 mg in a glass of white wine. Wine contains many phenolic substances, most of which originate in the grape berry. Wine phenolics include the non-flavonoids: hydroxycinnamates, hydroxybenzoates and the stilbenes; plus the flavonoids: flavan-3-ols, the flavonols, and the anthocyanins [65]. These phenols are partly absorbed from the gastrointestinal tract in animals and humans, metabolized in the intestinal cells and in the liver and excreted by the urine [66-67]. Metabolites are glucuronide or sulfate conjugates and methylated conjugates [68]. While polymeric condensed tannins and pigmented tannins constitute the majority of wine phenolics, their large size precludes absorption so their health effects are likely restricted to the gut [69]. The health effects of dietary polyphenols might be explained by both intact compounds and their metabolites formed either in the tissues or in the colon by the microflora [70-73].

Polyphenol effects

Polyphenols and their metabolites have many biological activities. They present antioxidant, antithrombotic, antiinflammatory and anticarcinogenic properties. Their mechanism of action can be explained by a direct antioxidant effect and by modification of some protein properties than produced changes in enzymes activities (inhibition or activation) and gene expression. Some of these effects are the following:

Antioxidant

Polyphenols are recognized as strong antioxidant and oxygen free radical scavengers [74-75]. Red wine and red wine phenols increase *in vitro* the resistance of human LDL against oxidative modification [76]. Several studies report an increased resistance to oxidative modification of human LDL after long-term consumption of red wine and red wine polyphenols [77-78]. In contrast, other studies report that red wine and a phenolic extract from red wine do not affect LDL oxidizability [79-80]. In an acute ingestion study in humans, postprandial LDL obtained after a meal consumed with ethanol was more

susceptible to metal-catalyzed oxidation than the homologous baseline LDL. On the contrary, postprandial LDL obtained after a meal consumed with wine, was as resistant as or more resistant to lipid peroxidation than fasting LDL [81]. Also the experimental meal taken with wine provoked a significant increase in the total plasma antioxidant capacity and in the plasma concentration of alpha-tocopherol and SH groups. In another study the supplementation of a meal with grape seed extract reduced the postprandial oxidative stress by decreasing the oxidants and increasing the antioxidant levels in plasma and, as a consequence, enhancing the resistance to oxidative modification of LDL [82].

In our intervention study in humans described before, to evaluate the effect of a MD, an OD and their supplementation with red wine, we found that the total antioxidant reactivity (TAR) was significantly higher in volunteers on MD compared to those on OD and wine intake increased TAR significantly in both groups. The OD group showed higher levels of oxidative DNA damage, measured as 8-hydroxydeoxyguanosine (8-OHdG) levels in blood leukocyte DNA and elevated protein damage markers, when compared with the MD group. Wine intake significantly decreased 8-OHdG in both diets, particularly in the OD, where the 8-OHdG levels decreased to values similar to those in the MD plus wine group. Wine intake induced an increase in plasma and urinary polyphenols. The results presented support the following conclusions: an OD induces oxidative stress; a diet rich in fruits and vegetables enhances antioxidant defenses; wine supplementation to an OD or a MD improved antioxidant defenses in both groups and counteracted the oxidative damage produced by the OD [83-84].

Antithrombosis

Polyphenols enhance in *in vitro* and *ex vivo* experiments, the generation of NO a platelet inhibitor and vasodilator [85-86]. Some studies were also performed to investigate the effect of wine polyphenols on experimental thrombosis in rats. In these studies NO was evaluated as a possible mediator of the effects [87]. Supplementation for 10 days with red wine or alcohol-free red wine, but not white wine or alcohol, induced a marked prolongation of bleeding time (BT) (258 +/- 13 *vs* 132 +/- 13 s in controls; $P < 0.001$) a decrease in platelet adhesion to fibrillar collagen (11.6 +/- 1.0 *vs* 32.2 +/- 1.3%; $P < 0.01$)

and a reduction in thrombus weight (1.45 +/- 0.33 *vs* 3.27 +/- 0.39 mg; $P < 0.01$). The effects of red wine were prevented by the NO inhibitor, L-NAME. In rats with diet-induced hyperlipidemia, alcohol-free red wine supplementation for one month reversed the prothrombotic effect of the diet, measured as delay in the thrombotic occlusion of an artificial prosthesis inserted into the abdominal aorta, without affecting the increased cholesterol and triglyceride levels [62]. Plasma antioxidant capacity measure as TRAP values, were significantly higher in animals receiving alcohol-free wine. These studies provides evidence that red wine modulates primary haemostasis and prevents experimental thrombosis in rats, independent of its alcohol content, by a NO-mediated mechanism.

Antiinflammation

Chemotaxis and accumulation of leukocytes in the arterial wall are considered to be critical events in the inflammation associated with atherosclerosis. Flavonoids were reported to inhibit adhesion of immune cells to endothelial cells. They down regulate gene expression of inflammatory mediators [88-89].

Anticarcinogenesis

It has been reported that dehydrated-dealcoholized red wine (wine solids) when consumed as part of a precisely defined complete diet, delays tumor onset in transgenic mice that spontaneously develop externally visible tumors without carcinogen pretreatment [90]. Particular phenol components of wine such as resveratrol and quercetin have shown an inhibitory effect on carcinoma cell proliferation [91-92]. Other studies have shown that red wine contains phytochemicals that inhibit aromatase activity *in vitro* and suppress aromatase-mediated breast tumor formation *in vivo* [93-94].

Some protein polyphenol interactions

Polyphenols especially flavonoids, have numerous effects caused by their interaction with different proteins. Middleton and collaborators reviewed the mammalian enzyme systems that are target of flavonoids [89]. Recent examples of these are the inhibition of aromatase activity [94] and angiotensin converting enzyme (ACE) [95] and activation of sirtuin, an enzyme associated to longevity [96].

Flavonoids and their *in vivo* metabolites may exert modulatory actions in cells through actions at protein kinase and lipid kinase signalling pathways. They have been reported to act on phosphoinositide 3-kinase (PI 3-kinase), Akt/protein kinase B (Akt/PKB), tyrosine kinases, protein kinase C (PKC), and mitogen activated protein kinase (MAP kinase) signaling cascades. Inhibitory or stimulatory actions at these pathways are likely to affect cellular function by altering the phosphorylation state of target molecules and by modulating gene expression [97].

Endothelial function

Normal endothelium-dependent vasomotor function or endothelial function (EF) is a physiological response, mediated by NO, which appears to play a key role in the prevention or reduction of the risk of atherosclerosis. Endothelial dysfunction is associated with risk factors for coronary heart disease such as hypercholesterolemia, hypertension, cigarette smoking, hyperhomocysteinemia and diabetes mellitus. Red wine polyphenols activate tyrosine kinases, increase cytosolic free calcium and stimulate a Ca(2+)-dependent release of NO in bovine aortic endothelial cells [98].

Moderate red wine intake improves endothelial function evaluated as flow-mediated dilation of the brachial artery [99]. Recalling our study on diets and wine supplementation we showed that in the absence of wine, there is a reduction of endothelial function with OD when compared to the MD ($P = 0.014$). This loss of endothelial function is not seen when both diets are supplemented with wine ($P = 0.001$). These effects are attributed to oxidative stress associated with an OD, and to the elevated plasma antioxidant capacity associated with wine consumption and the MD. Wine polyphenols protect NO from oxidation.

Acute red wine consumption produces an increase in coronary flow-velocity reserve in human volunteers. This finding suggests that some polyphenols may have potent vasorelaxing effects on coronary microvessels during hyperaemia [100].

Red wine polyphenol extract enhances eNOS expression and increases NO production in human umbilical vein endothelial cells (HUVECs) [101-102].

Red wines strongly inhibit the synthesis of endothelin-1 (ET-1), a vasocontrictor peptide that decreases endothelial function. Wine poyphenols decreased ET-1 release and transcription in bovine aortic endothelial cells [103]. ET-1 release induced by oxLDL is inhibited by quercetin [104].

Conclusion

The experimental results presented in this review do not support the frequently held conclusion that ethanol is the main active component, independent of antioxidants, when considering the biological consequences of moderate alcoholic beverage consumption. Quite clearly, effects such as those shown for haemostasis, or those to be expected when HDL levels increase, are related to polyphenols or to an efficient antioxidant system, respectively, which wine could provide. For example, if the new paradigm to explain the protective role of elevated HDL levels requires the participation of eNOS, it is obvious that antioxidants will be necessary to protect the NO generated, a well known requirement for NO mediated effects. This consideration could also lead to the idea that ethanol, consumed together with antioxidant rich foods, would be biologically safer than drinking alone. Equally, the different results obtained in epidemiological studies that consider alcohol consumption, could well be related to the dietary habits of those populations, in particular the type, quantity and opportunity of antioxidant-rich food consumption, as well as prooxidant food consumption.

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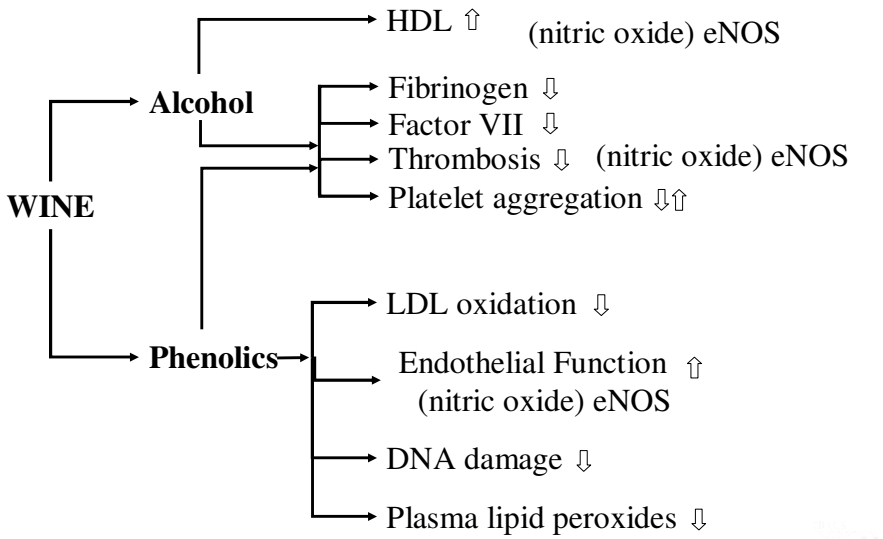

Figure:

Favorable changes in CV risk factors shown in moderate wine drinkers.

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